Soluble Fas as a programmed cell death marker before and after antioxidant vitamins supplement in type 1 diabetes and high-risk children

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Background/Aim
Considerable evidence indicates that increased oxidative stress and induction of apoptosis signaled through the Fas pathway appear to play an important role in the pathogenesis of autoimmune diabetes. The present study aimed to detect the soluble Fas (sFas) as apoptotic marker and the total antioxidant capacity (TAC) in type 1 diabetes (T1D) and high-risk group children and whether it is altered by antioxidant vitamin supplement.

Patients and methods
Forty-five participants were included in the study and divided into three groups: group 1 comprised 15 children with new onset diabetes; group 2 included 15 diabetic children with long-standing diabetes; and group 3 comprised 15 individuals of patient's relatives. Serum levels of sFas and TAC were measured and compared between groups before and after antioxidant vitamin supplementation.

Results
The highest level of sFas was found in group 2 (2196.7 ± 579 pg/ml), however, with no statistical significance; after vitamins supplementation, its level showed significant decrease to reach 1156.6 ± 460.8 pg/ml ($P = 0.01$). Similar tendency of serum Fas decrease was observed in the group of relatives after vitamins supplementation (2088.3 ± 396.5 vs. 1426.7 ± 140.9, $P < 0.01$). TAC was significantly lower in group 2 than in the other two groups, and it showed a significant increase after vitamin intake (0.29 ± 0.06 vs. 0.40 ± 0.05 μmol/l, $P < 0.05$).

Conclusion
One month of treatment with antioxidants vitamins supplement increased the antioxidant activity in long-standing T1D children and resulted in significant reduction in sFas level, suggesting the importance of this therapeutics in reducing apoptosis changes in children with T1D.

Keywords:
apoptosis, soluble Fas, total antioxidant capacity, type 1 diabetes mellitus

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Introduction

Type 1 diabetes (T1D) is an autoimmune disease resulting from the destruction of insulin-producing pancreatic β-cells by autoreactive T cells. This destruction occurs silently and progressively and may stay undetected for many years [1]. Diabetes mellitus is characterized by increased production of reactive oxygen species (ROS), sharp reduction in antioxidant defense, and altered cellular redox status. Hyperglycemia, a key clinical manifestation of diabetes mellitus, not only generates more ROS, but also attenuates antioxidative mechanisms by scavenging enzymes and substances [2]. Oxidative stress is an imbalance that favors the production of ROS over antioxidant enzymes [3]. Despite the considerable evidence for an imbalance in oxidative stress and antioxidant power in diabetes, the proof is still needed that this imbalance is harmful and causally involved in the worsening of either metabolic control and/or diabetic vascular complications. The multiple sources of radical generation as well as of plasma and cellular antioxidants have raised considerable hope that antioxidant therapy represents a very promising avenue to treat a series of diseases [4]. Antioxidants such as vitamin C, vitamin E, and β-carotene have been considered as ideal supplements against oxidative stress and its complications [5].

Indeed, when defense mechanisms cannot prevent the accumulation of ROS, there is an increase in cellular damage proteins, lipids, and nucleic acids. Accumulation of such injury ultimately leads to cell death through necrotic or apoptotic mechanisms [6]. Proteins secreted by the damaged cells including soluble Fas (sFas) circulate in small but detectable amounts. Fas is generated by alternative mRNA splicing capable of encoding a sFas molecule lacking the transmembrane domain [7]. Fas (Apo-1 or CD95) is a cell-surface receptor that transduces apoptotic signals from Fas ligand (FasL). The Fas/FasL system

is a key regulating system responsible for activation of apoptosis in various cell types including cellular constituents of the vessel wall [8,9]. Immune-mediated apoptosis specifically triggered through Fas is believed to be central to the pathogenesis of autoimmune diabetes because this apoptotic mechanism destroys islet b-cells in both animal models of diabetes and the human type 1 disease. In contrast, animals engineered to prevent Fas-mediated cytotoxicity of islet cells do not develop disease [10].

Much attention has been given to the ability of free radicals to induce apoptosis; hence, the study was designed to detect the apoptotic changes and the total antioxidant capacity (TAC) in T1D and high-risk group children and to find out whether it is altered by antioxidant vitamin supplement.

Patients and methods

The study included 45 participants divided into three groups: group 1 comprised 15 patients with recently diagnosed T1D (with disease duration<2 months), including six male patients and nine female patients; group 2 comprised 15 patients with long-standing T1D (more than 6 months duration), including eight male patients and seven female patients; and group 3 included 15 healthy individuals with family history of T1D, including six male individuals and nine female individuals.

Approval from National Research Center Medical Ethical Committee was obtained before this study.

Patients were examined within the same period for sFas as an apoptosis marker and TAC level, and they were subjected to detailed history taking and full clinical examination.

Diabetic patients included in the study were diagnosed according to the criteria of ISPAD clinical practice guidelines (2009) [11]; they were recruited from the Pediatric Diabetic Clinic of Children Hospital, Ain Shams University.

Blood samples were collected from the patients after overnight fasting. All samples were immediately centrifuged and stored at -80°C until the time of assay.

sFas was determined using sFas enzyme-linked immunosorbent assay kit (Research & Diagnostics Systems, Minneapolis, Minnesota, USA), with a sensitivity of 20 pg/ml in serum.

Total antioxidant status of plasma was determined by ferric reducing antioxidant power assay, whereby, at low pH, there is reduction of ferric tripyridyl triazine ($Fe3^{+}$-TPTZ) complex (Sigma Aldrich, St Louis, Missouri, USA) to ferrous form, which has an intense blue color that can be monitored by measuring the absorbance at 593 nm using spectrophotometer. It is directly related to the combined or total reducing power of the electron-donating antioxidants present in the reaction mixture. The results were expressed as μmol/l.

All the studied participants were supplemented with 100 mg/day vitamin E and 50 000 IU of vitamin A for 1 month. After that month of supplementation, all the parameters mentioned above were studied again.

Glycated hemoglobin was determined by automated high-performance liquid chromatography.

Statistical analysis was carried out with statistical package for social science program, version 10 for Windows (SPSS Inc., Chicago, Illinois, USA). The quantitative data were presented in the form of mean and SD. The Kruskal–Wallis test was used for group comparison. Nonparametric relationships were examined with the Mann–Whitney *U*-tests. Paired *t*-test was used to compare values before and after treatment. *P* values less than 0.05 were deemed statistically significant.

Results

The main characteristics of the studied group are shown in Table 1. The mean age of the group of newly diagnosed (group 1) and of the group of relatives (group 3) was comparable with each other (5.7 ± 3.6 vs. 6.3 ± 3.8 years). However, long-standing diabetic patients (group 2) were older in average; their mean age was 12.8 ± 4.0 years, which differed significantly in comparison with the other groups. All participants in group 3 had a positive family history of type 1 diabetes mellitus (T1DM). Disease duration ranged from 0.5 to 2 months in group 1 and from 2 to 10 years in group 2.

Table 1 Clinical characteristics of diabetic patients and normal relatives

Parameter	Group 1 (new onset diabetics) (*n* = 15)	Group 2 (long-standing diabetics) (*n* = 15)	Group 3 (relatives) (*n* = 15)
Mean age ± SD (years)	5.7 ± 3.6	12.8 ± 4.02*	6.3 ± 3.8
Female/male (*n*)	9/6	7/8	9/6
Fasting blood glucose (mg/dl)	226.6 ± 112	226.9 ± 55.4	95.75 ± 12.98*
HbA_{1c} (g%)	6.5 ± 0.9	9.02 ± 1.2*	5.1 ± 0.4

HbA_{1c}, glycated hemoglobin A_{1c}; *Significant difference than each group at $P < 0.05$.

Comparison between the diabetic groups with respect to their glycemic control revealed a significant increase in glycated hemoglobin in the old diabetic patients compared with newly diagnosed patients (9.02 ± 1.2 vs. 6.5 ± 0.9 mg%, P = 0.03). However, there was a nonsignificant difference between old and new cases with respect to fasting blood glucose (226.9 ± 55.4 vs. 226.6 ± 112, P = 0.3).

Assessment of sFas was performed to the studied groups before antioxidant vitamins administration. The highest level was found in long-standing diabetic patients (2196.7 ± 579 pg/ml); however, no significant differences were found between the three studied groups. Reassessment of sFas was performed after vitamins supplementation; in long-standing diabetic patients (group 2), serum Fas levels (2196.7 ± 579 pg/ml) showed significant decrease (1156.6 ± 460.8 pg/ml, P = 0.01). Similar tendency of serum Fas decrease was observed in the group of relatives after vitamins supplementation (2088.3 ± 396.5 vs. 1426.7 ± 140.9, P < 0.01), but there was no difference determined in sFas levels among the group of newly diagnosed following antioxidant intake (Table 2).

Comparison of TAC between the studied groups showed a significant difference, as it was lower in long-standing diabetic patients than in the other two groups; reassessment after vitamins supplementation revealed a significant increase in long-standing diabetic patients (0.29 ± 0.06 vs. 0.40 ± 0.05 μmol/l, P < 0.05), but there were no differences in the other two groups presupplementation and postsupplementation (Table 3).

Table 2 Serum soluble Fas level in diabetic patients and their relatives before and after treatment with antioxidant

Parameter	Group 1 (new onset diabetics)	Group 2 (long-standing diabetics)	Group 3 (relatives)
sFas pregroup (pg/ml)	1620 ± 751.5	2196.7 ± 579	2088.3 ± 396.5
sFas postgroup (pg/ml)	1283.3 ± 387.7	1156.6 ± 460.8*	1426.7 ± 140.9*

All data are expressed as mean ± SD; sFas, soluble Fas; *Significant difference than sFas pregroup at P < 0.05.

Table 3 Serum total antioxidant capacity in diabetic patients and their relatives before and after treatment with antioxidant

Parameter	Group 1 (new onset diabetics)	Group 2 (long-standing diabetics)	Group 3 (relatives)
TAC pregroup (μmol/l)	0.37 ± 0.07	0.29 ± 0.06	0.39 ± 0.03
TAC postgroup (μmol/l)	0.4 ± 0.05	0.40 ± 0.05*	0.37 ± 0.09

All data are expressed as mean ± SD; TAC, total antioxidant capacity; *Significant difference than TAC pregroup at P < 0.05.

Discussion

Oxidative stress plays a role in the pathological processes ongoing in diabetic patients; excessive oxidant stress has adverse effects on islet cell survival and function and accelerates complications in target organs and tissues. Antioxidant therapy may play a critical role in reducing morbidity and mortality in diabetes. However, at this time, studies do not allow us to determine quantitatively to what extent oxidative stress plays a role in deterioration of islet function and worsening of the complications [12]. We assessed the levels of serum Fas as an apoptosis cell death marker and of the TAC to determine the antioxidant defense in patient with T1D compared with other group of healthy patient's relatives, with further evaluation of the effect of vitamins supplementation in combating oxidative stress and cellular damage.

sFas has been detected in human serum. A previous study indicated that a dysregulation in Fas-mediated apoptosis was involved in both the insulitis process and the pancreatic cell death; in the pancreas of newly diagnosed T1D patients, the Langerhans islets were infiltrated by FasL-expressing T lymphocytes, whereas the few remaining cells were strongly Fas positive [13].

In the current study, sFas level was higher in long-standing diabetic patients compared with the other two groups but with no statistical significance. Recently, Mahfouz *et al*. [14] found that sFas serum levels were significantly increased in both diabetic groups as compared with normal controls, but the levels were significantly higher in patients with long duration of diabetes when compared with those with short duration of diabetes (P < 0.05). In accordance, an Egyptian study on 40 T1D patients revealed a significant increase in Fas percentage expression in T1D patients in comparison with controls, and they explained that the findings of immunological, inflammatory, and metabolic signals leading to b-cell apoptosis were increased in diabetic patients and they proposed that these signals converge toward a common b-cell death signaling pathway [15]. In addition, Andrikoula and colleagues found a lower serum sFas level in recent onset T1D patients (P < 0.01) than in groups of long-standing and normal controls. They postulated the decreased sFas levels in the first stage of T1DM to the downregulation of this soluble protein, in order not to prevent Fas–FasL interaction during β-cell apoptosis, with an interruption to this downregulation through subsequent stages of the disease, when complete destruction of pancreatic cells occurs. The previous findings suggest that apoptosis signaled through the Fas pathway appears to play an important role in the pathogenesis of autoimmune diabetes. The regulation

of this pathway in the islet cells may alter the clinical expression of the disease [10].

Mechanism of cell death induced by oxidative stress is under intense investigation by many groups [16,17]. There is growing evidence that excess generation of highly reactive free radicals, largely due to hyperglycemia, causes oxidative stress, which further exacerbates the development of diabetes complications. Overproduction and/or insufficient removal of these free radicals result in vascular dysfunction, damage to cellular proteins, membrane lipids, and nucleic acids [18]. Thus, chronic hyperglycemia at the onset of diabetes may be associated with increased apoptosis. Cells manifest potent antioxidant defenses against ROS, including detoxifying enzymes and exogenous free radical scavengers (vitamins). In healthy individuals, antioxidants form the body's primary defense against ROS. They scavenge ROS before they cause damage to various biological molecules and prevent oxidative damage from spreading; by interrupting the free radical chain reaction, antioxidants donate an electron to the free radical [19,20].

In agreement with our finding, a defective total antioxidant defense in patients with T1DM has previously been reported in many studies [21–23]. However, there are also studies showing no significant difference in antioxidant status between patients and healthy controls [24,25].

The reduced plasma antioxidant capacity present in diabetic patients has been postulated to an increased consumption of distinct antioxidant components (e.g. intracellular glutathione) or to primarily low levels of antioxidant substances (flavonoids, carotenoids, vitamins E and C) [26]. To ensure a substantial antioxidant effect, it is necessary to supply two distinct factors simultaneously acting in reciprocal oxidation and reduction and assuring in that way their sufficient regeneration [27]. Protection of free radical scavengers might help to maintain higher levels of antioxidants under treatment with this concentrate, and several components of the product (carotenoids, vitamins E and C) show important protective role against oxidative stress [28].

This is in accordance with the study by Varvařovská and colleagues who achieved improvement of metabolic control in response to vitamin E supplementation over 1 year in a group of T1D children compared with nonsupplemented patients and found reduced markers of oxidative stress substantially when compared with nonsupplemented diabetic children. They recommended the early use of antioxidants to decrease ROS with subsequent improvement of metabolic control and prevention of microvascular complication in diabetic patients [29].

Karataş *et al.* [30] had proposed use antioxidant vitamins to block formation of free radicals, and hence prevent development of diabetes and its complications. In the current study, nonsignificant differences of TAC after vitamins supplementation were found in newly diagnosed patients; however, there was a significant increase in the group with long-standing diabetes. On the basis of previous reports, it seems that the longer is the treatment, the better are the results. In contrast, this study evaluated the role of antioxidant vitamins E and A in reducing apoptosis; there was a significant decrease in serum sFas levels in the group of long-standing diabetic patients as well as in the group of relatives, which provide an evidence that apoptosis can be modulated by antioxidants in T1DM patients and in their first-degree relatives who are considered as the vulnerable group.

Conclusion

Targeted therapeutics that makes islets less susceptible to programmed cell killing would in the end be attractive strategies to prevent T1D especially for the vulnerable population. On the basis of data presented, we conclude that 1 month of treatment with vitamins supplement increased the antioxidant activity in long-standing T1D children and resulted in significant reduction in sFas level. Our findings must be supported by further studies with larger sample size.

Acknowledgements

Conflicts of interest
None declared.

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